# Lambda Gene Map

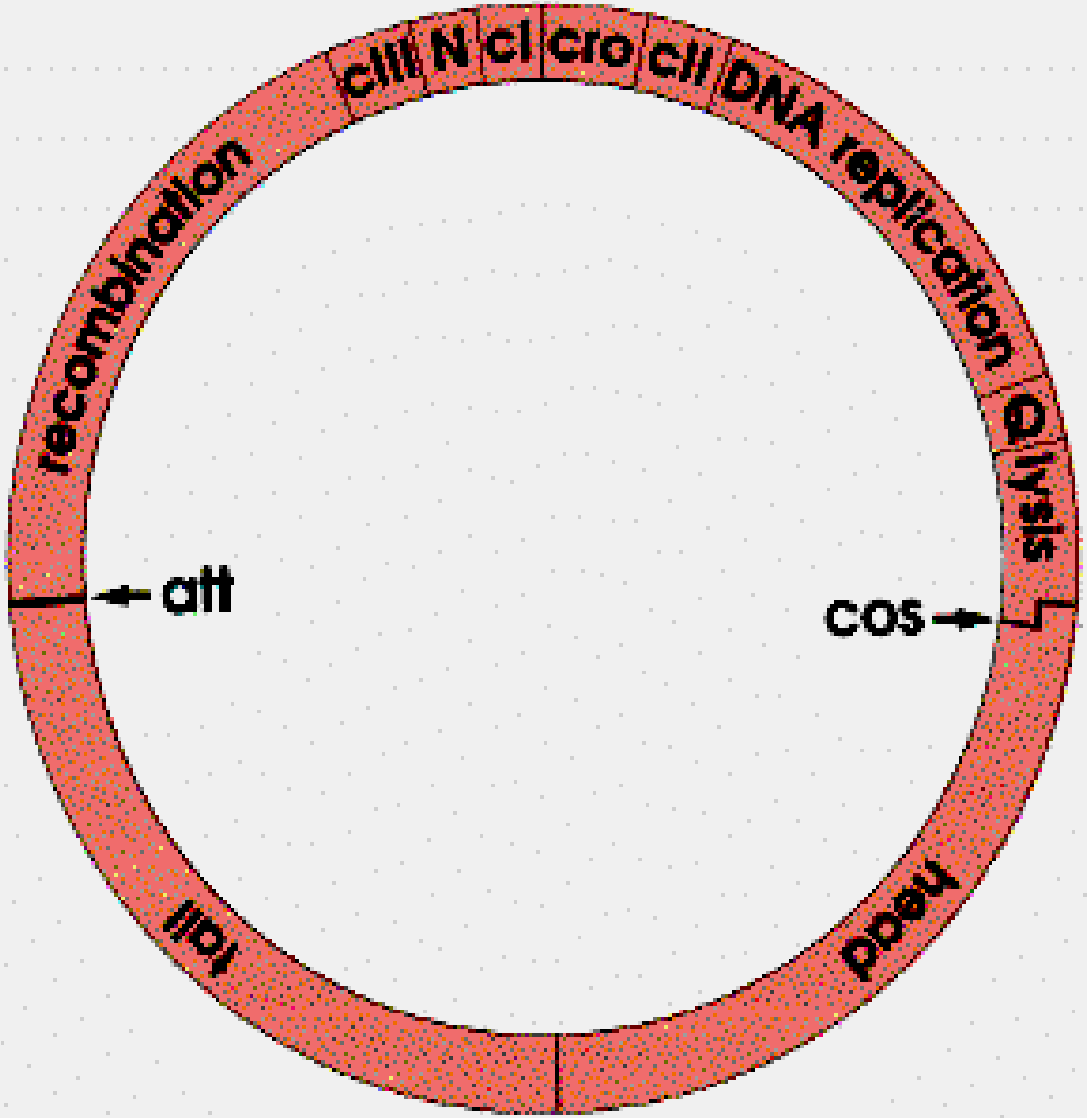



Figure 3.1. The λ chromosome. In general, genes of related function are grouped together. The genes within each of these groups are, as a rule, regulated coordinately. On this map six control genes are named individually, as are two sites, *att* (attachment site) and cos (cohesive ends).

# Gene Expression Patterns

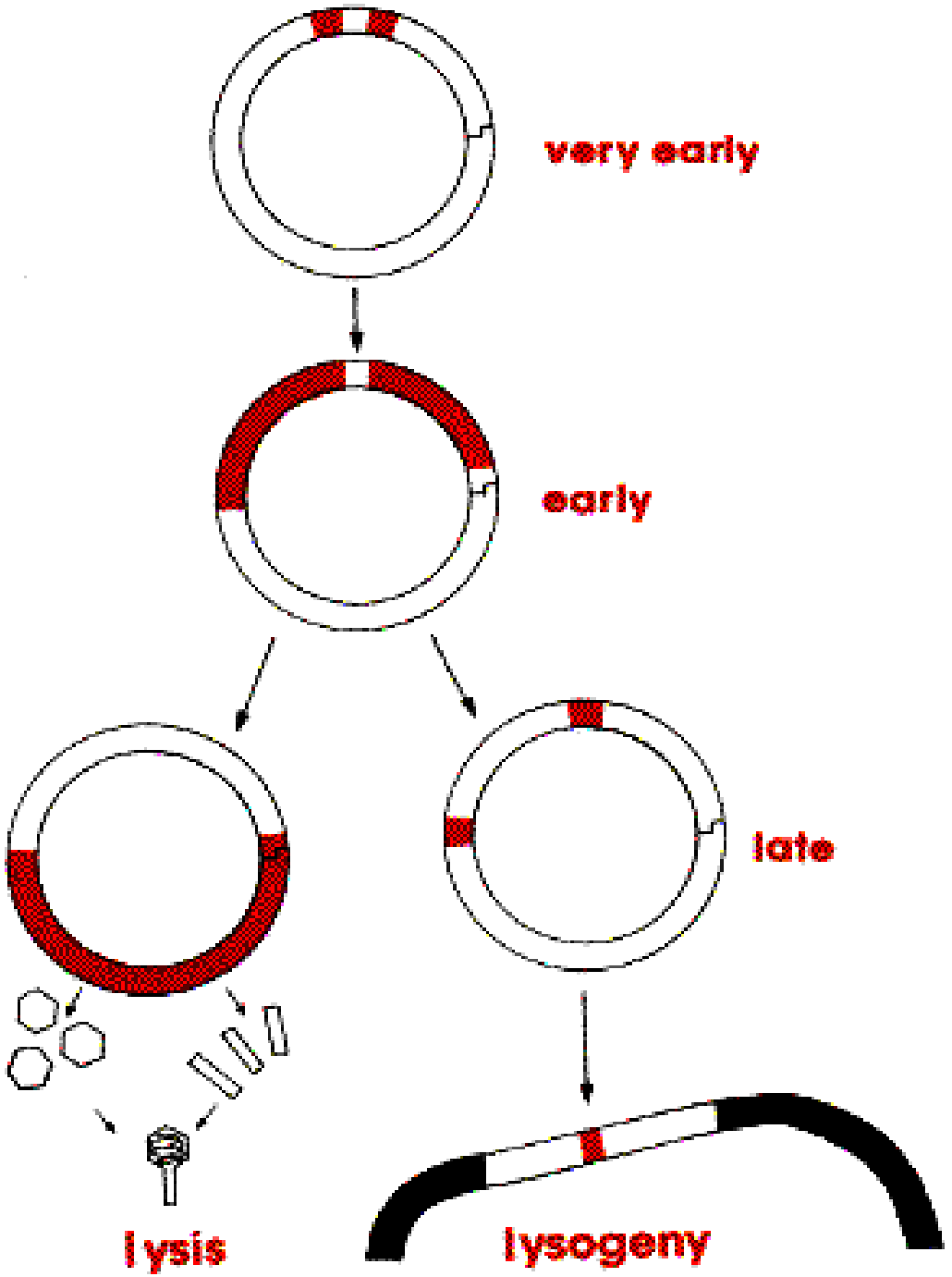



Figure 3.3. Patterns of gene expression. The genes shown in red are on at each of the indicated stages of growth. Genes of related function are turned on and off together. These coordinately regulated genes lie in contiguous blocks except at the late stages of lysogenic growth where only two genes, *cI* and *int*, are active.

# Very Early Transcription

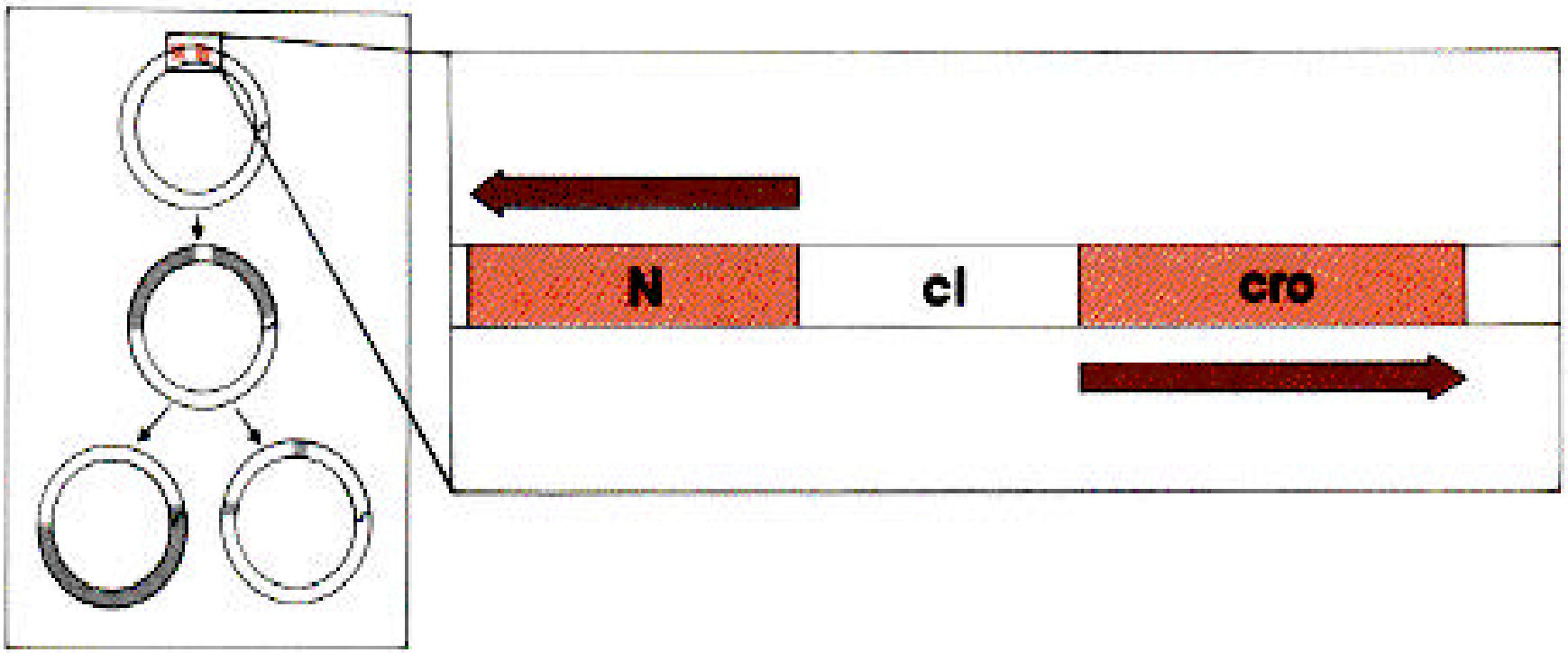



Figure 3.5. Very early events. Very early after infection the *E. coli* RNA polymerase transcribes genes *N* and *cro* from different strands of the DNA.

# Early Events of N Protein

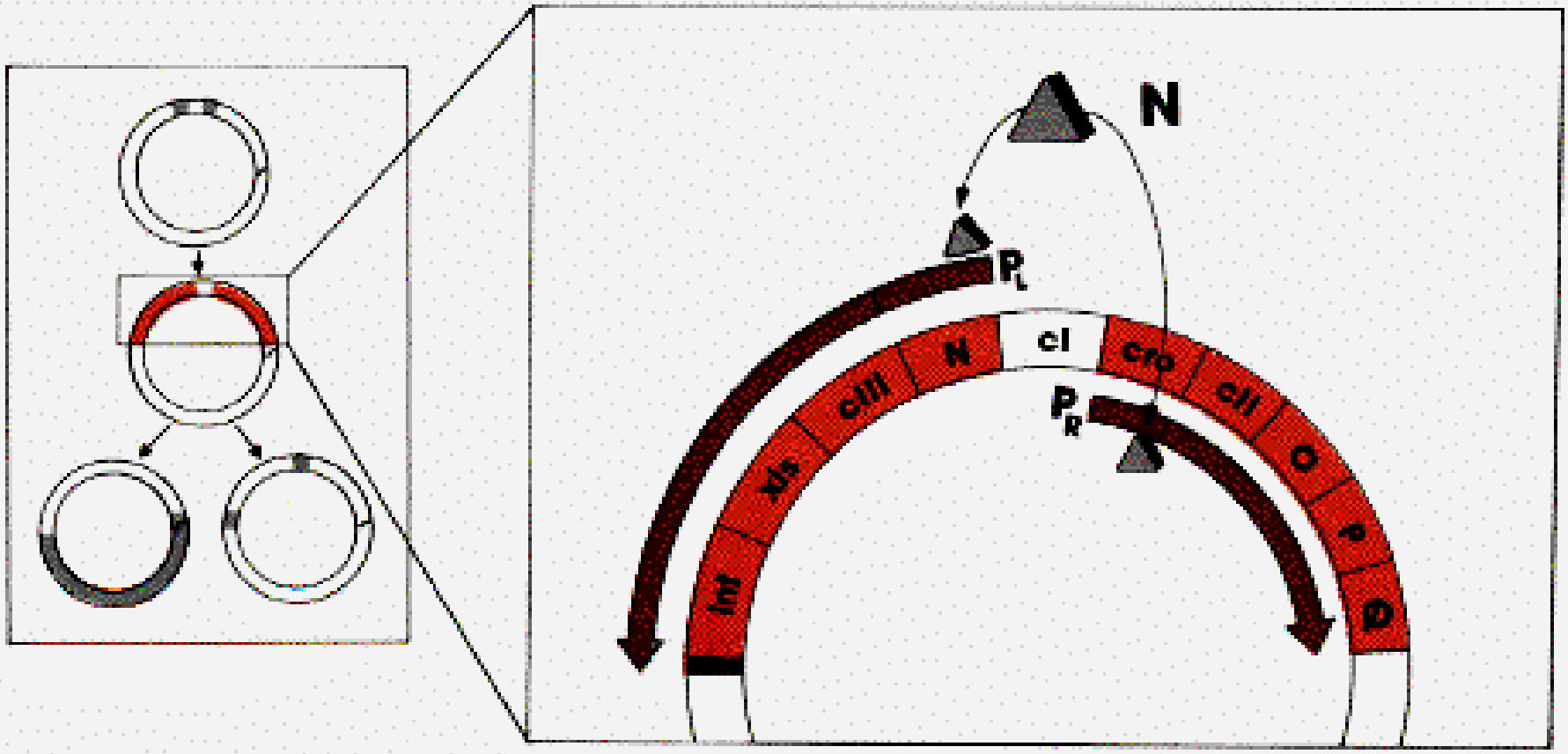



Figure 3.6. Early events. N protein turns on the early genes to the left of *N* and to the right of *cro*. The pyramid representing N protein is shown hovering near the beginning of the leftward mRNA, but further downstream in the case of the rightward mRNA. This is explained in the text.

# Action of N

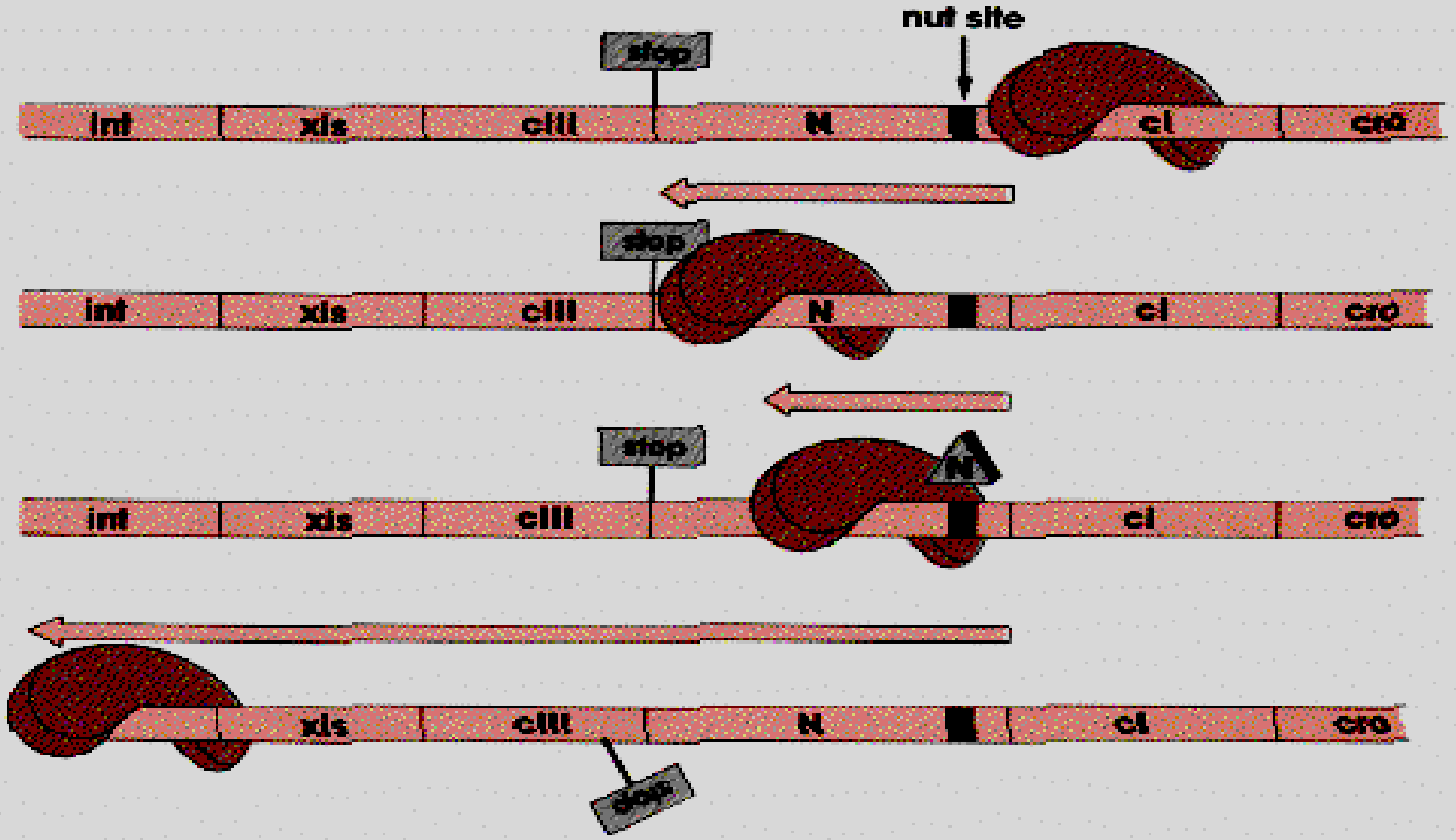



Figure 3.7. The action of N. If no N protein is present polymerase ignores the *Nut* site and falls off the DNA, releasing the mRNA, when it reaches the stop signal. But in the presence of N polymerase becomes a juggernaut as it passes over *Nut* and ignores the stop signal.

# Late Lytic Events

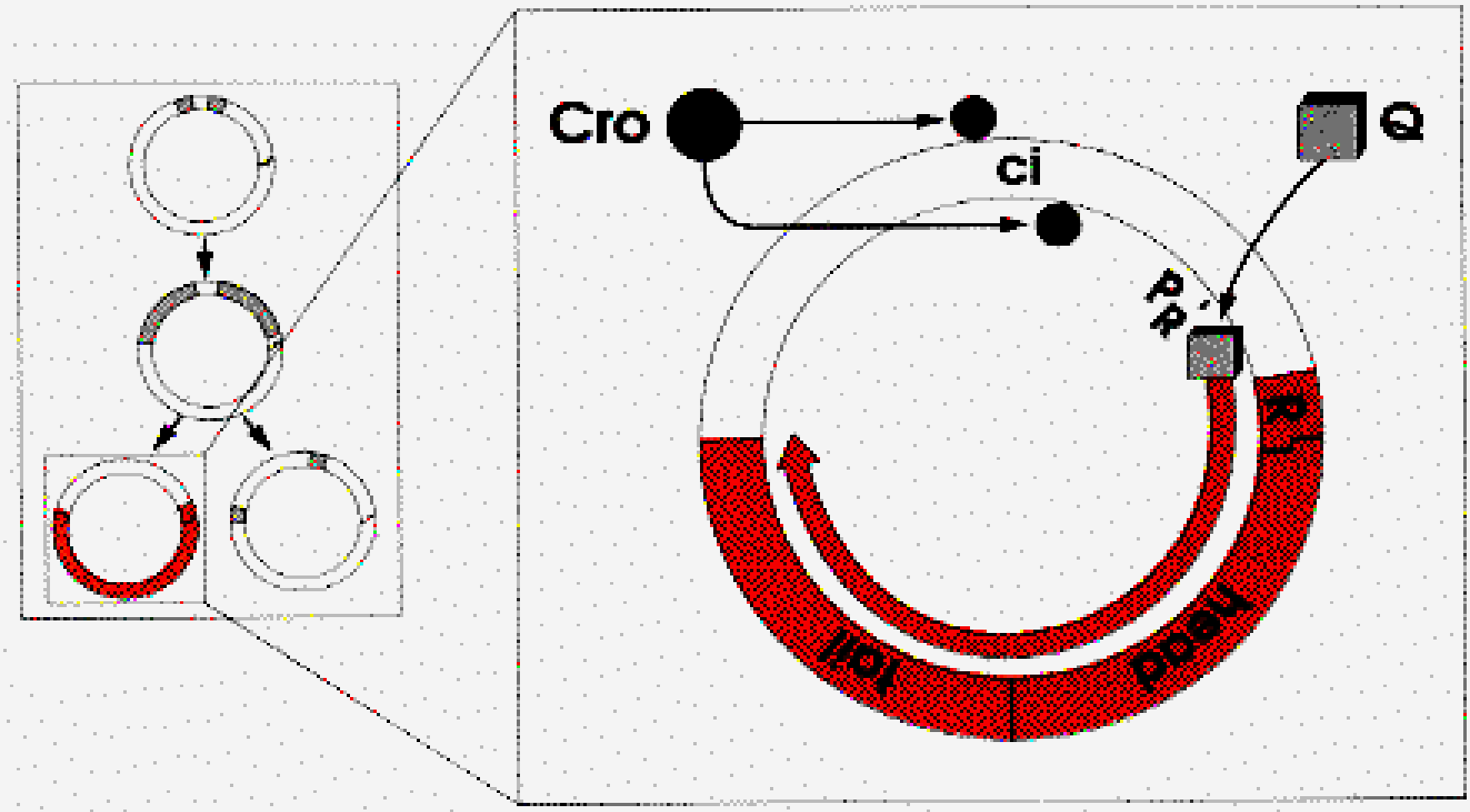



Figure 3.8. Late lytic events. The site Q recognizes, *Qut*, lies very near the beginning of the long transcript that initiates at $P_R'$. The Q-modified polymerase transcribes the late genes into a single long transcript.

# Late Lysogenic Events

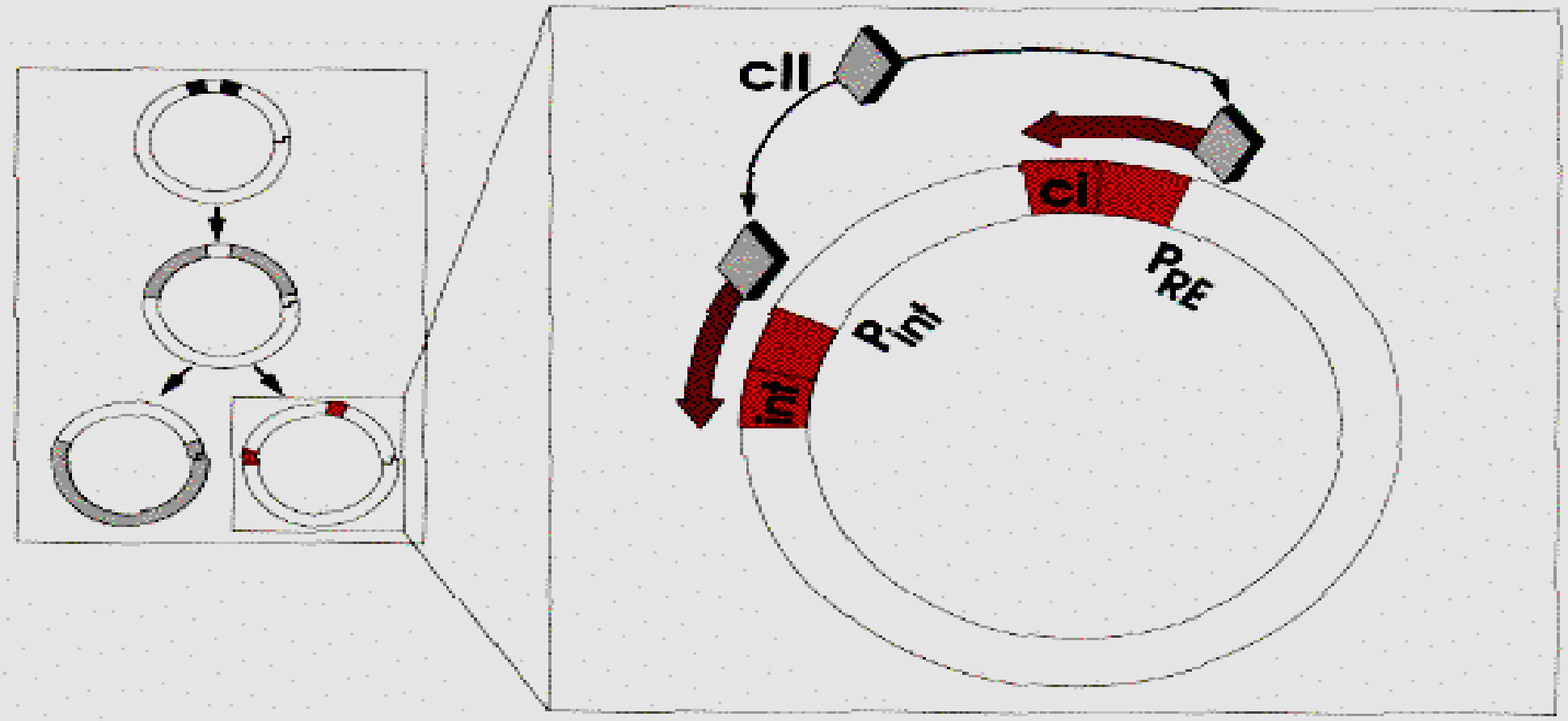



**Figure 3.9.** Late events in establishing lysogeny. CII protein directs transcription of the two genes needed for finally establishing lysogeny. The early genes are probably still on at this stage but these transcripts have been omitted from the figure, as has been the anti-Q transcript.

# Lysis-Lysogeny Decision

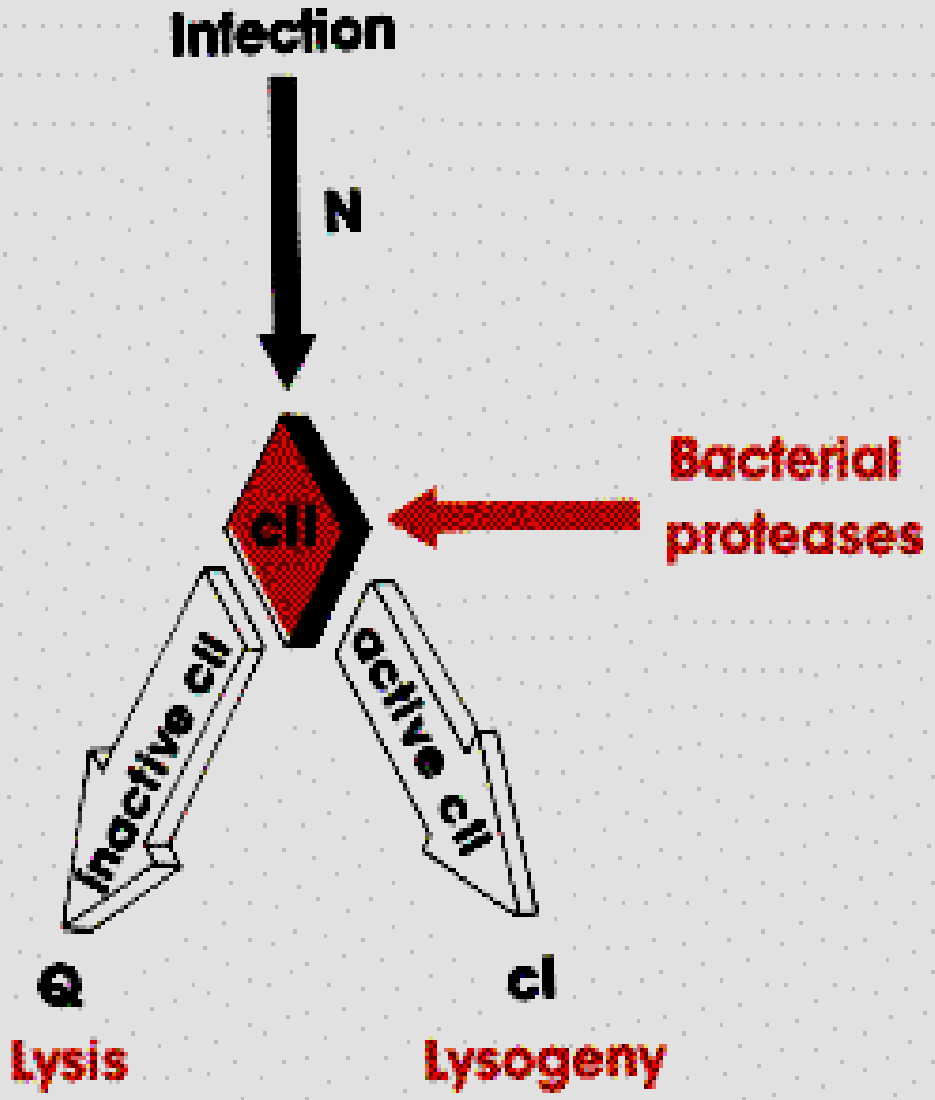



Figure 3.10. The lysis-lysogeny decision. Host proteases regulate the level of activity of CII protein. Although CIII protein is not shown here, the host factors may exert their effects by working on CIII, which protects CII. It is likely that other host proteins regulate translation of the CII mRNA as well.

# CII Stimulated Transcription

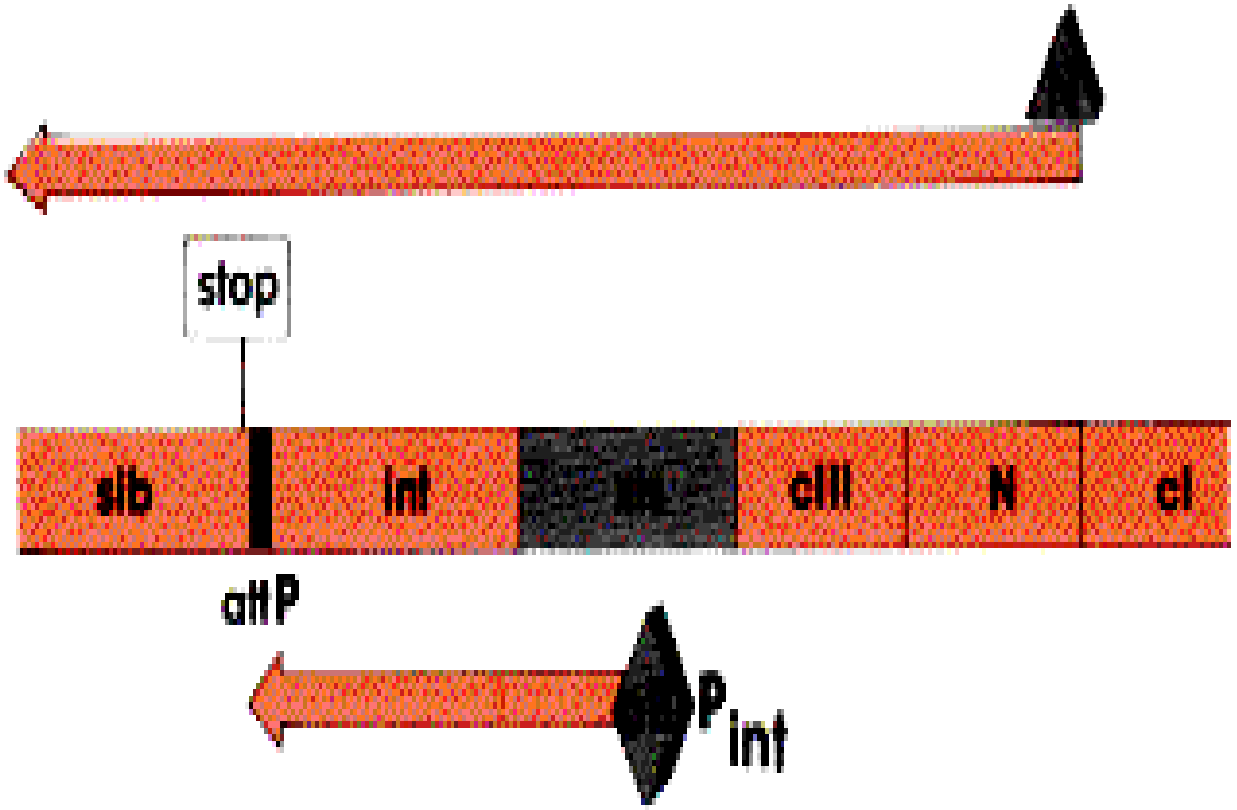



Figure 3.11. CII-stimulated transcription of *int*. The promoter for the *int* gene, $P_{int}$, lies within the *xis* gene. Therefore Int but not Xis production is stimulated by CII.

# Lytic Growth Retroregulation

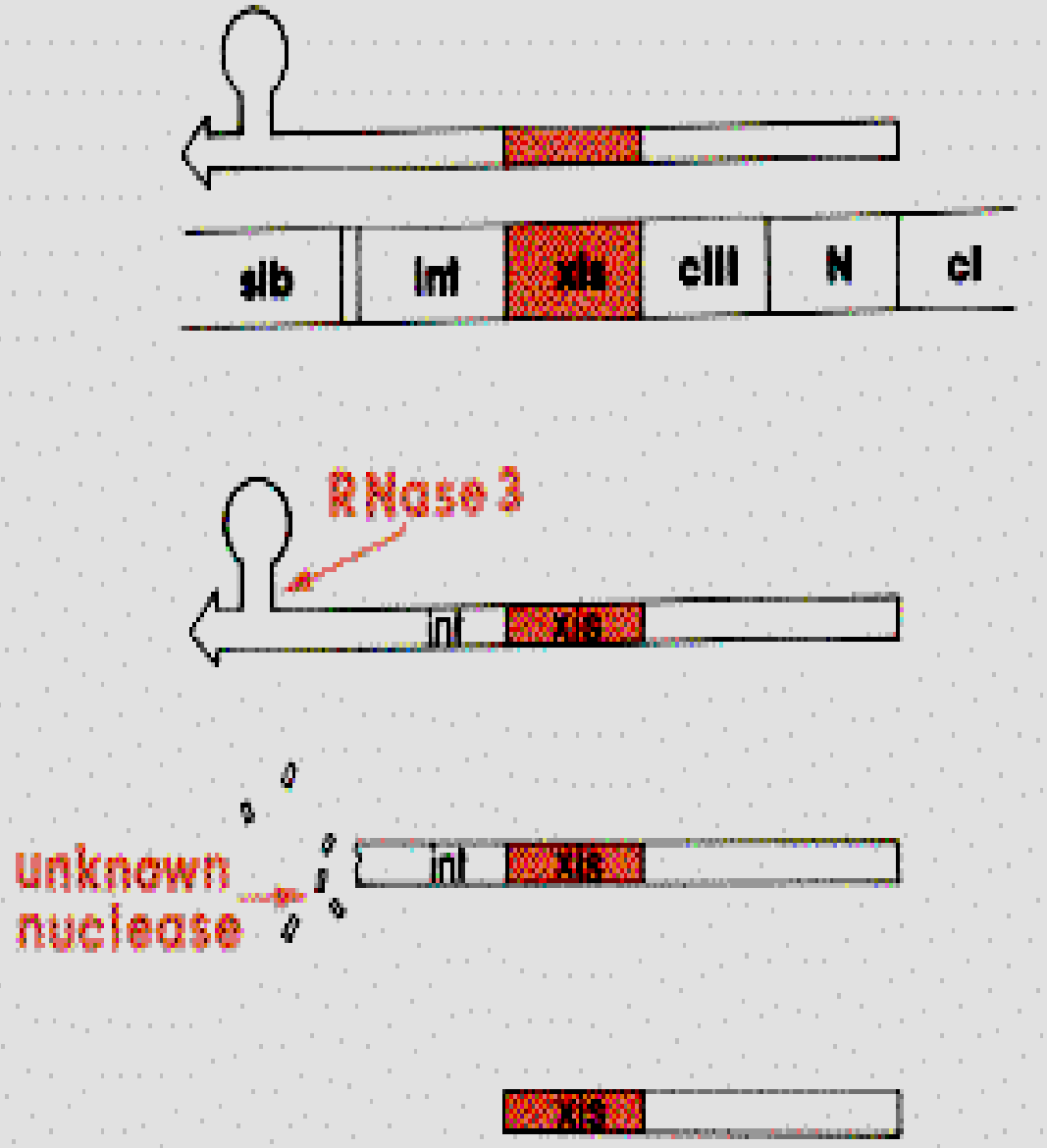



Figure 3.12. Retroregulation. The mRNA copy of sib forms a hairpin that attracts the bacterial enzyme RNase III, which cleaves the hairpin. Other bacterial RNase molecules then chew the mRNA, beginning at the cleavage site.

# Integration and Gene Order

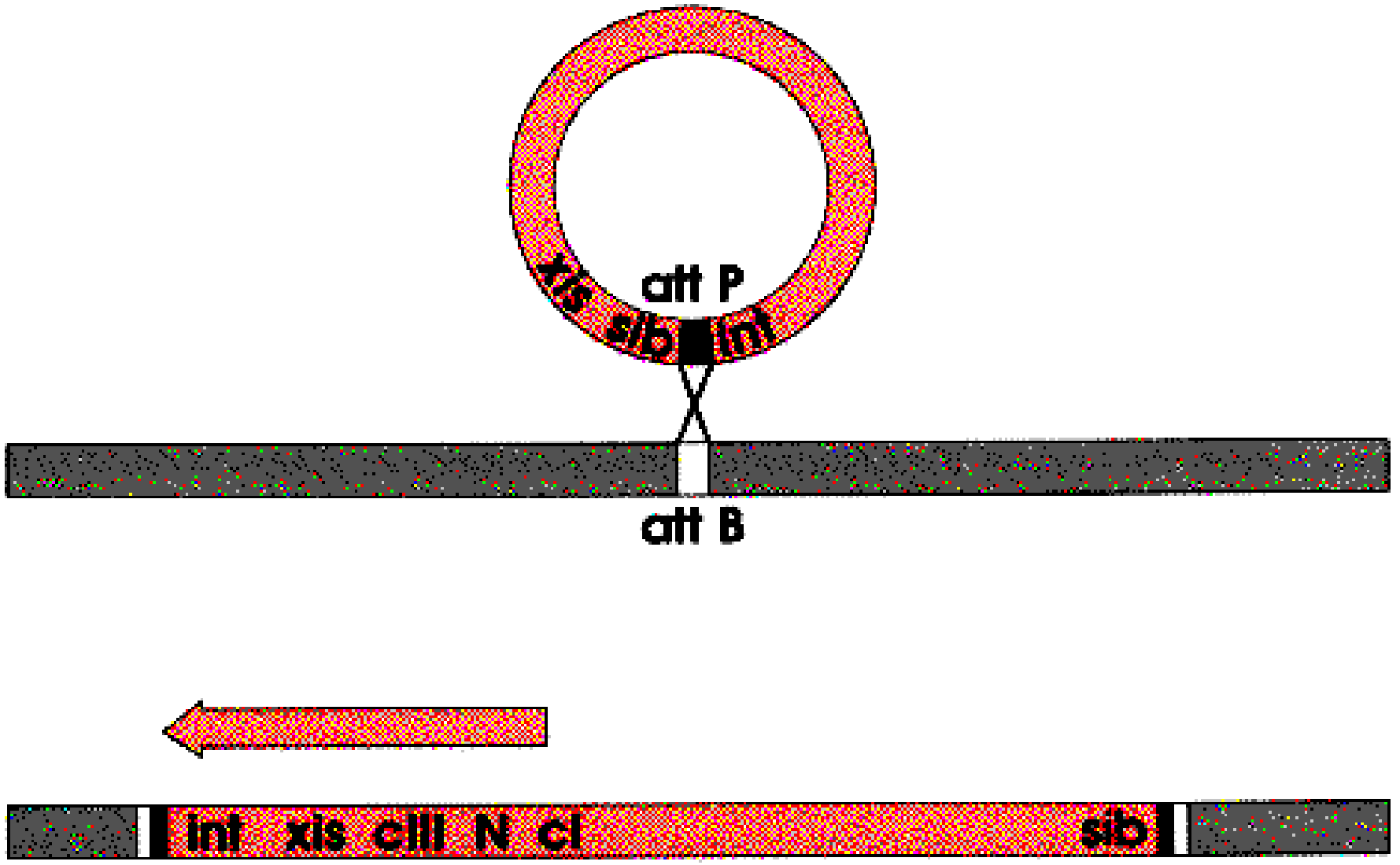



Figure 3.13. Integration and gene order. Integration (recombination at *att*) has separated *sib* from *int*.

# Three Right Operators

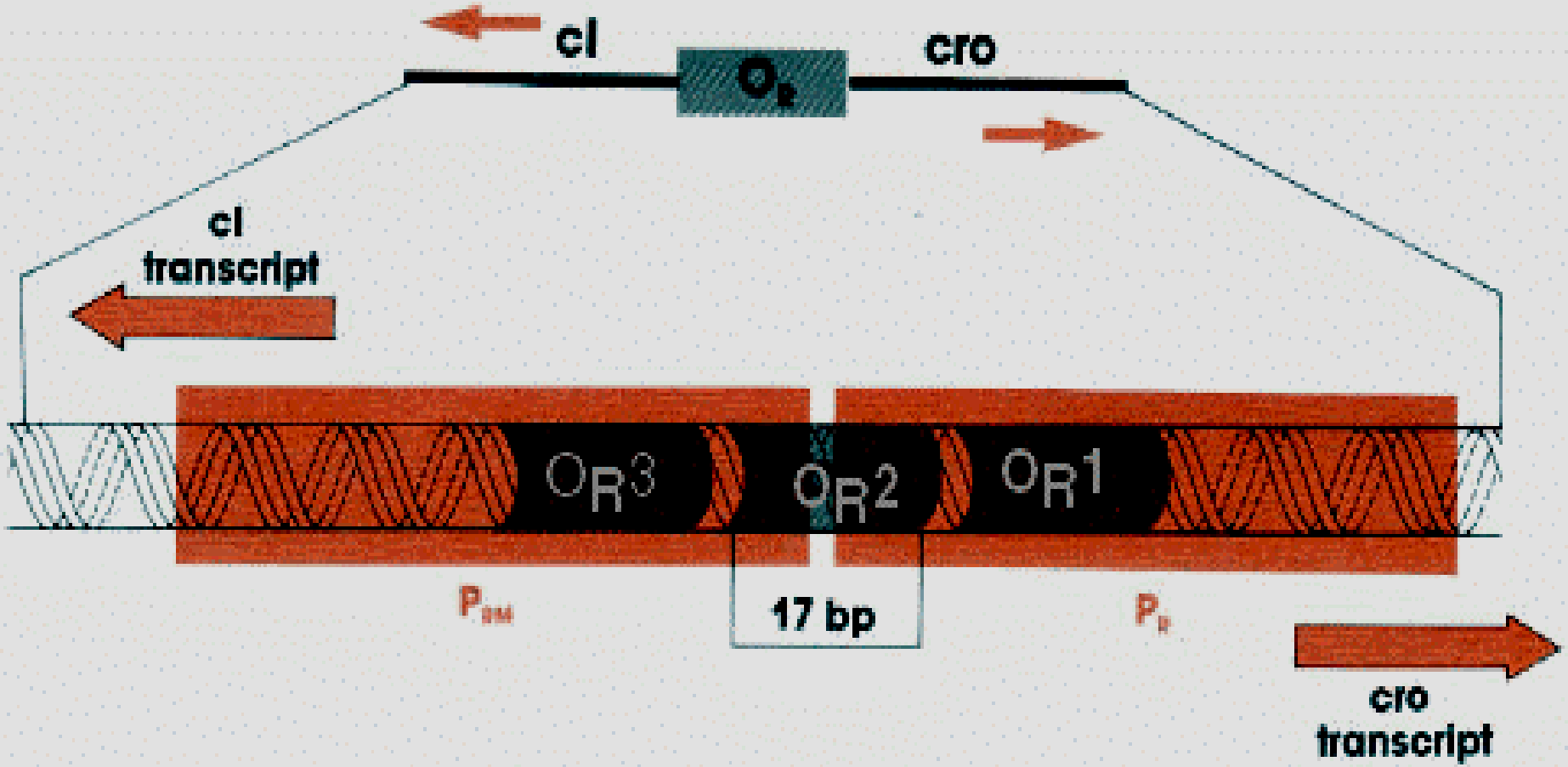



Figure 1.4. A short segment of the λ DNA molecule. Two back-to-back promoters ($P_{RM}$ and $P_R$) send polymerase traveling in opposite directions—leftward to transcribe the repressor gene (*cI*) and rightward to transcribe the *cro* gene. The tripartite right operator ($O_R$) overlaps the two promoters. Each of the three parts of the operator is called an operator site.

# Operator Mutations

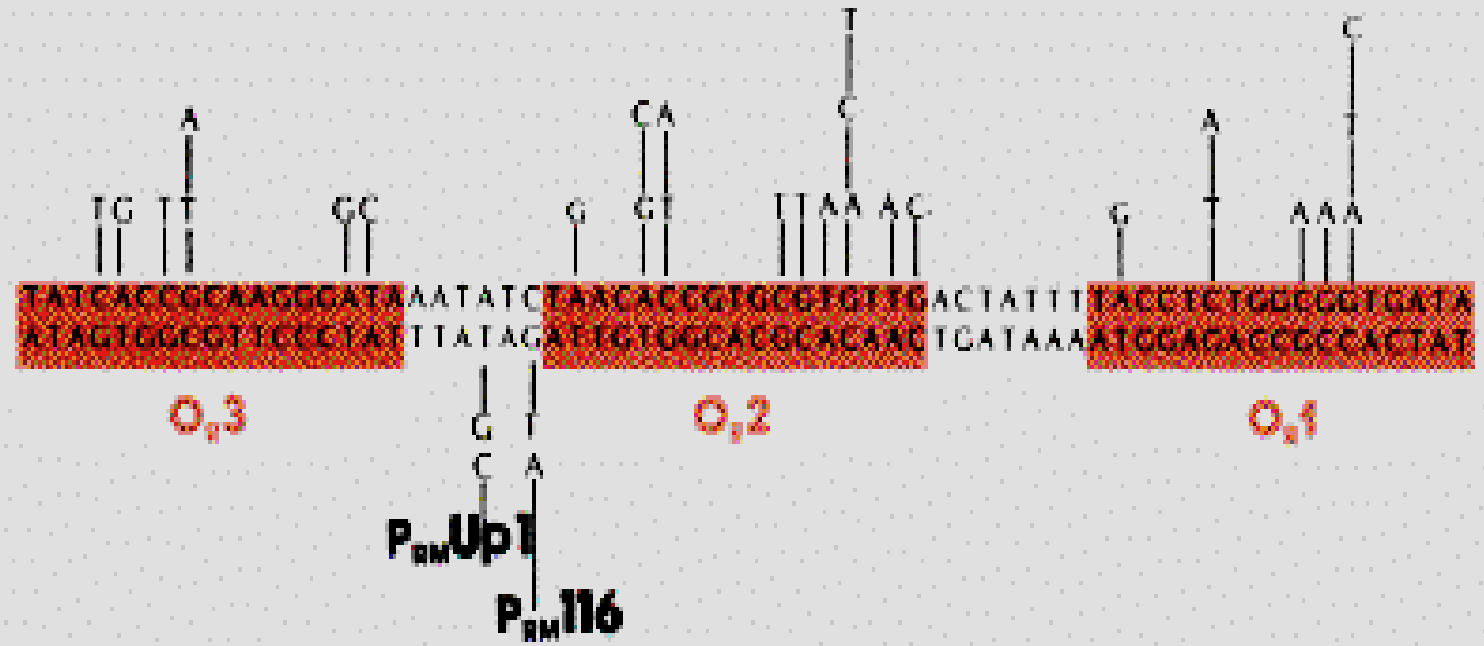



Figure 4.17. Mutations in and around $O_R$. This array of mutations should be compared with the sequences listed in Table 2.2. Mutations at position 3 decrease predominantly Cro but not repressor binding, and mutations at 8 and 9 decrease predominantly repressor but not Cro binding. Mutations at the remaining positions in the operator decrease the binding of both proteins. $P_{RM}Up-1$ renders $P_{RM}$ active in the absence of repressor and $P_{RM}116$ inactivates $P_{RM}$.

# Promoter - Operator Relationship

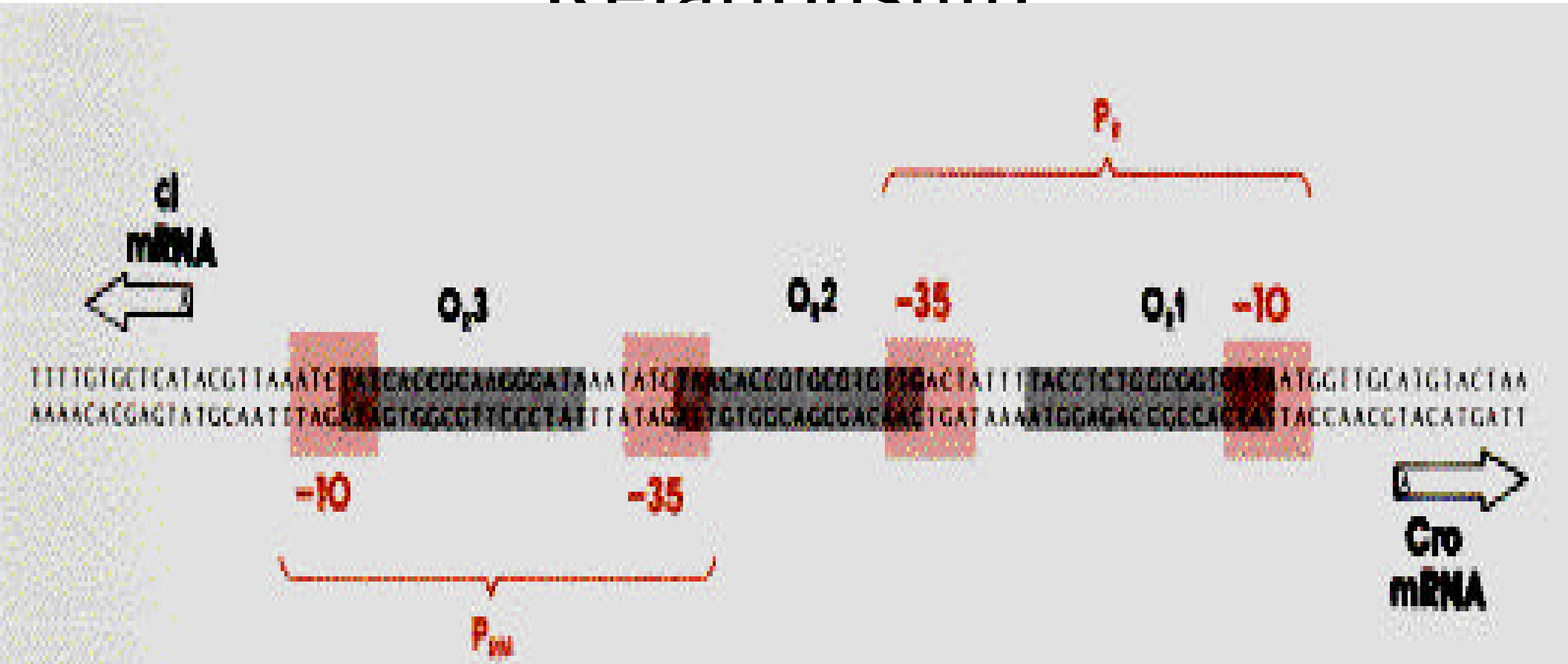
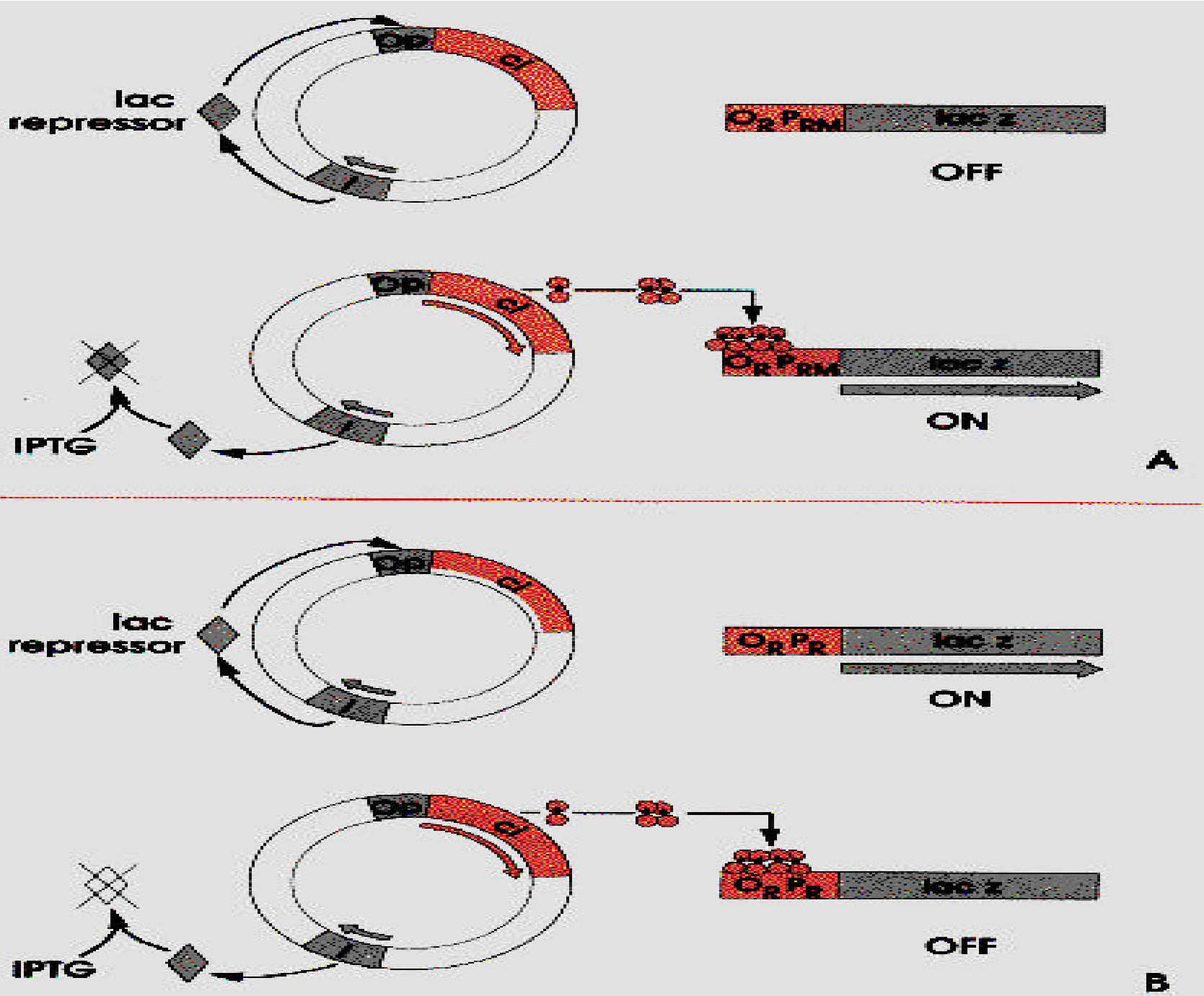



Figure 2.16. Linear relationship between promoter and operator sites around $O_R$. Some base pairs serve dual functions in the region between *cI* and *cro*. For example, three of the base pairs of $O_R2$ form part of the −35 region of $P_R$.

# Experimental Approach



# Regulation of Repressor Synthesis

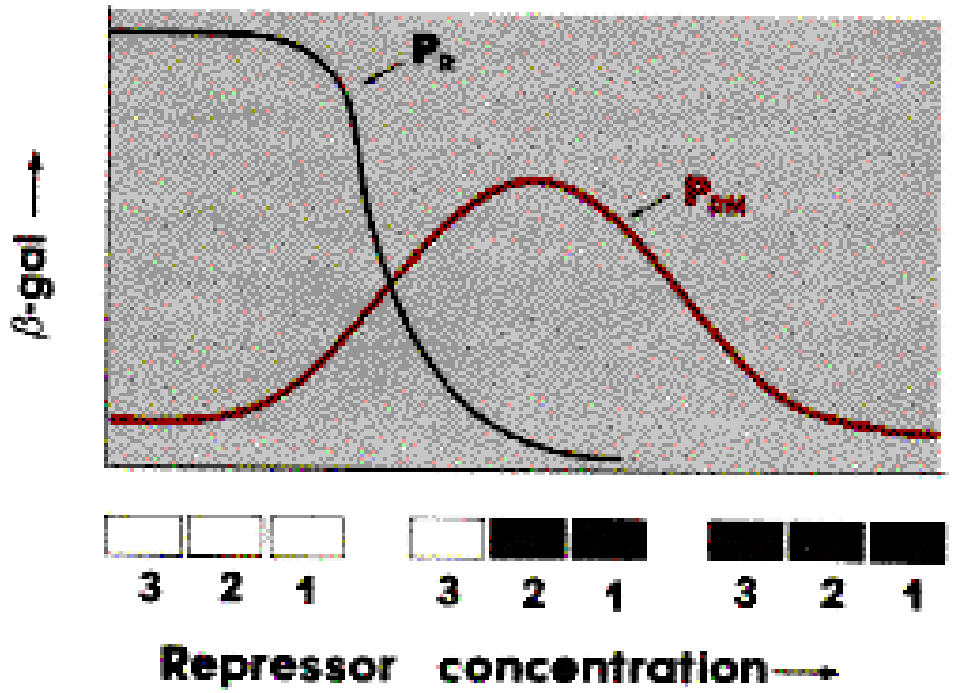



Figure 4.20. Regulation by repressor *in vivo*. This graph summarizes the results of an experiment using the two cells of Figure 4.19. For each case, β-galactosidase production was measured as a function of IPTG concentration. The midpoints of the repression and activation curves occur at the same IPTG concentration and hence at the same repressor concentration. The repressor level required to maximally activate $P_{RM}$ is near that found in a lysogen. The boxes show the states of each operator site at the various repressor concentrations. When black they are occupied by repressor.

# Repressor Binding

**Table 4.2.** The effect of a repressor dimer bound to a single site in $O_R$. In the absence of repressor, $P_{RM}$ is off (unstimulated) and $P_R$ is on, as shown in the top row.

| $P_{RM}$ | $O_R3$ | $O_R2$ | $O_R1$ | $P_R$ |
|---|---|---|---|---|
| off | | | | on |
| off | | | X | off |
| on | | X | | off |
| off | X | | | on |

# Repressor Dimer Model

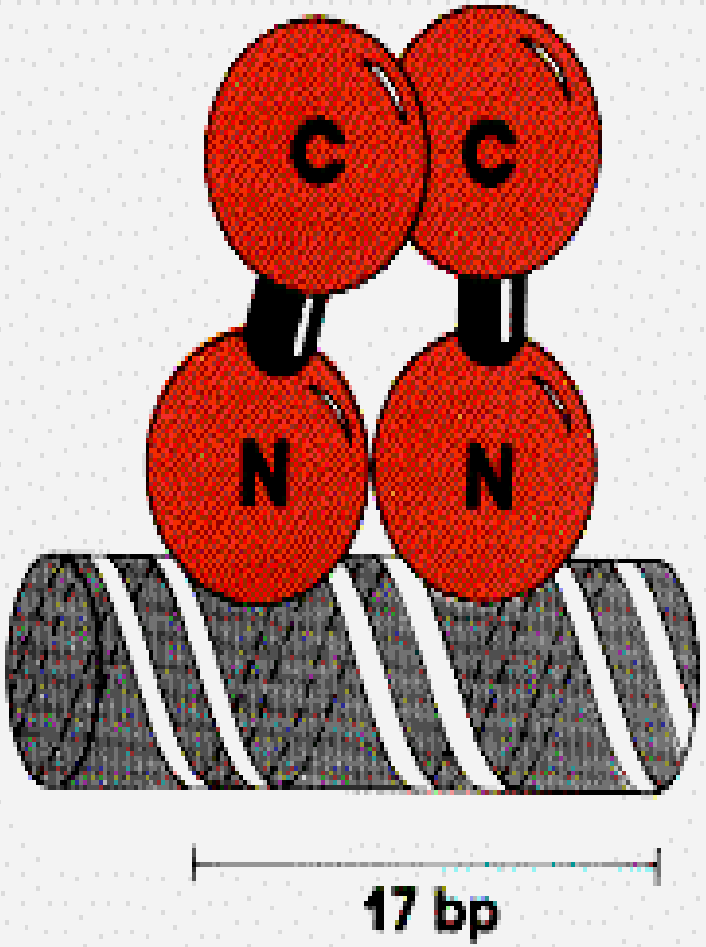



Figure 1.9. A repressor dimer bound to one 17 base pair operator site. Each amino domain is centered on a segment of the major groove, a point we return to in Chapter 2.

# Repressor Binding Order

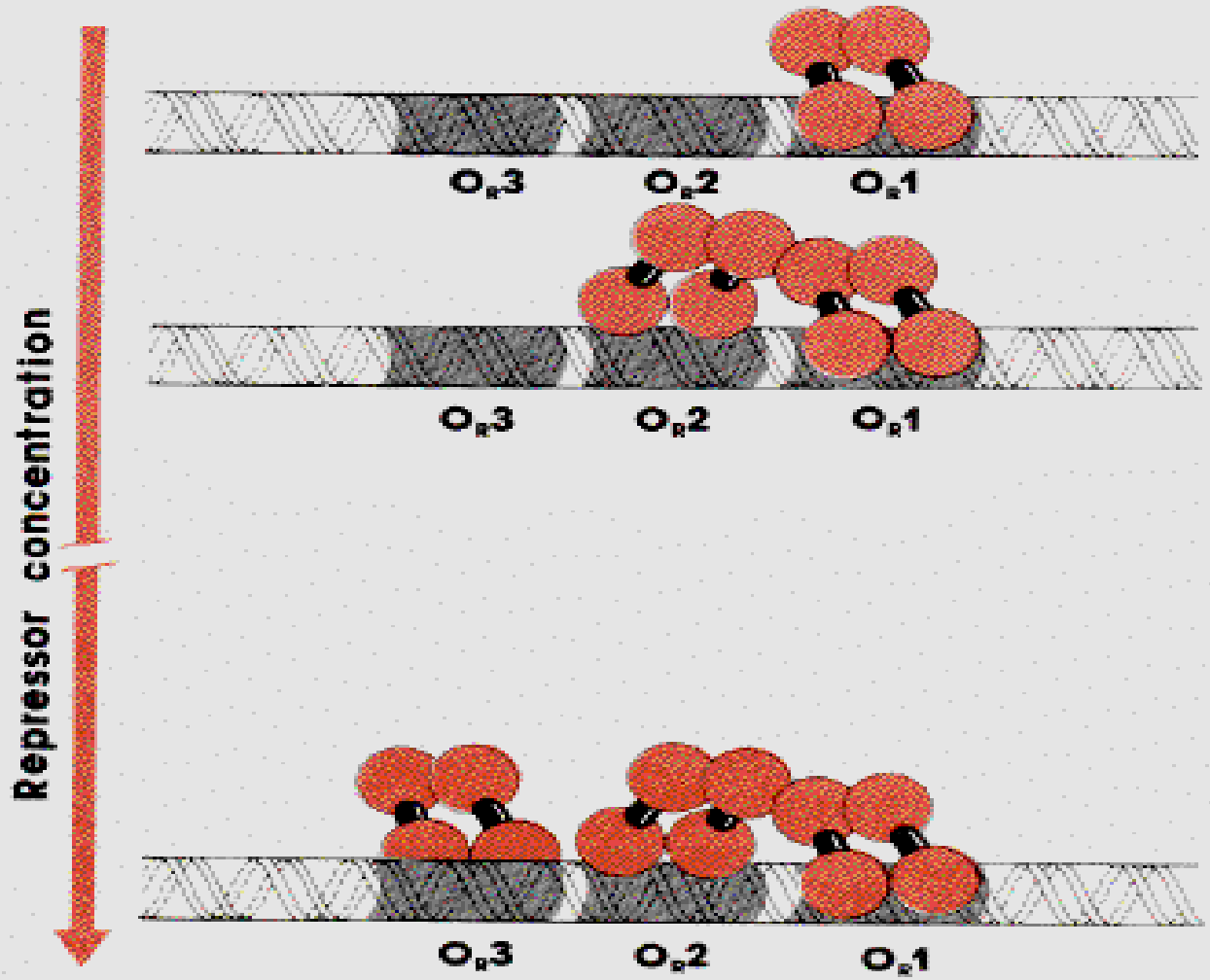
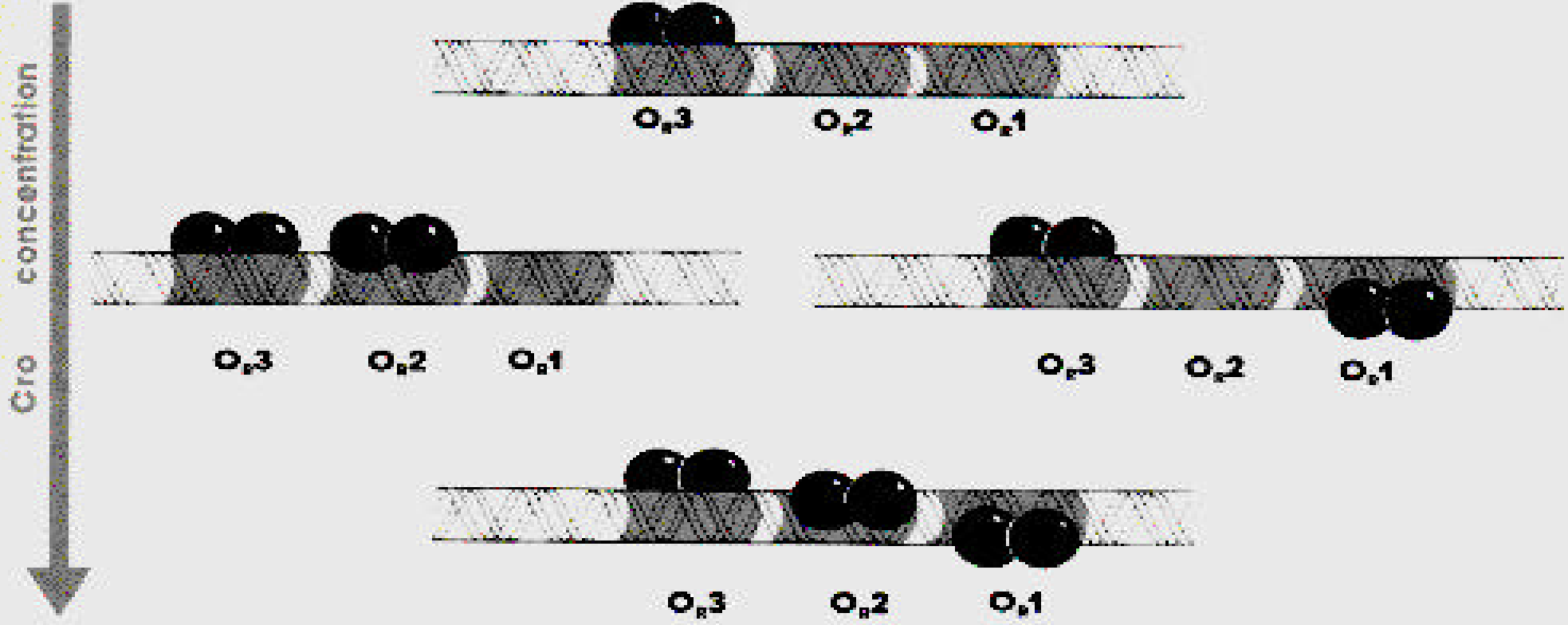



Figure 1.16. Repressor binding to the three sites in $O_R$. $O_R1$ binds repressor about 10 times more tightly than does $O_R2$ or $O_R3$, so repressor first binds to $O_R1$. A second repressor very quickly binds to $O_R2$, but $O_R3$ continues to bind weakly, and is filled only at higher repressor concentration.

# Cro Binding to Operators


Figure 1.22. Cro bound to $O_R$. Cro dimers bind independently to each site in the tripartite operator.



# Sequence of Operators

**Table 2.1.** The six operator sites recognized by λ repressor and Cro.

| | |
|---|---|
| $O_L1$ | T A T C A C C G C C A G T G G T A<br>A T A G T G G C G G T C A C C A T |
| $O_R1$ | T A T C A C C G C C A G A G G T A<br>A T A G T G G C G G T C T C C A T |
| $O_L2$ | T A T C T C T G G C G G T G T T G<br>A T A G A G A C C G C C A C A A C |
| $O_L3$ | T A T C A C C G C A G A T G G T T<br>A T A G T G G C G T C T A C C A A |
| $O_R2$ | T A A C A C C G T G C G T G T T G<br>A T T G T G G C A C G C A C A A C |
| $O_R3$ | T A T C A C C G C A A G G G A T A<br>A T A G T G G C G T T C C C T A T |

The sites are listed in the order of their intrinsic affinities for a λ repressor dimer. The central base pair, the axis of symmetry, is shown in red.

# Helix-Turn-Helix

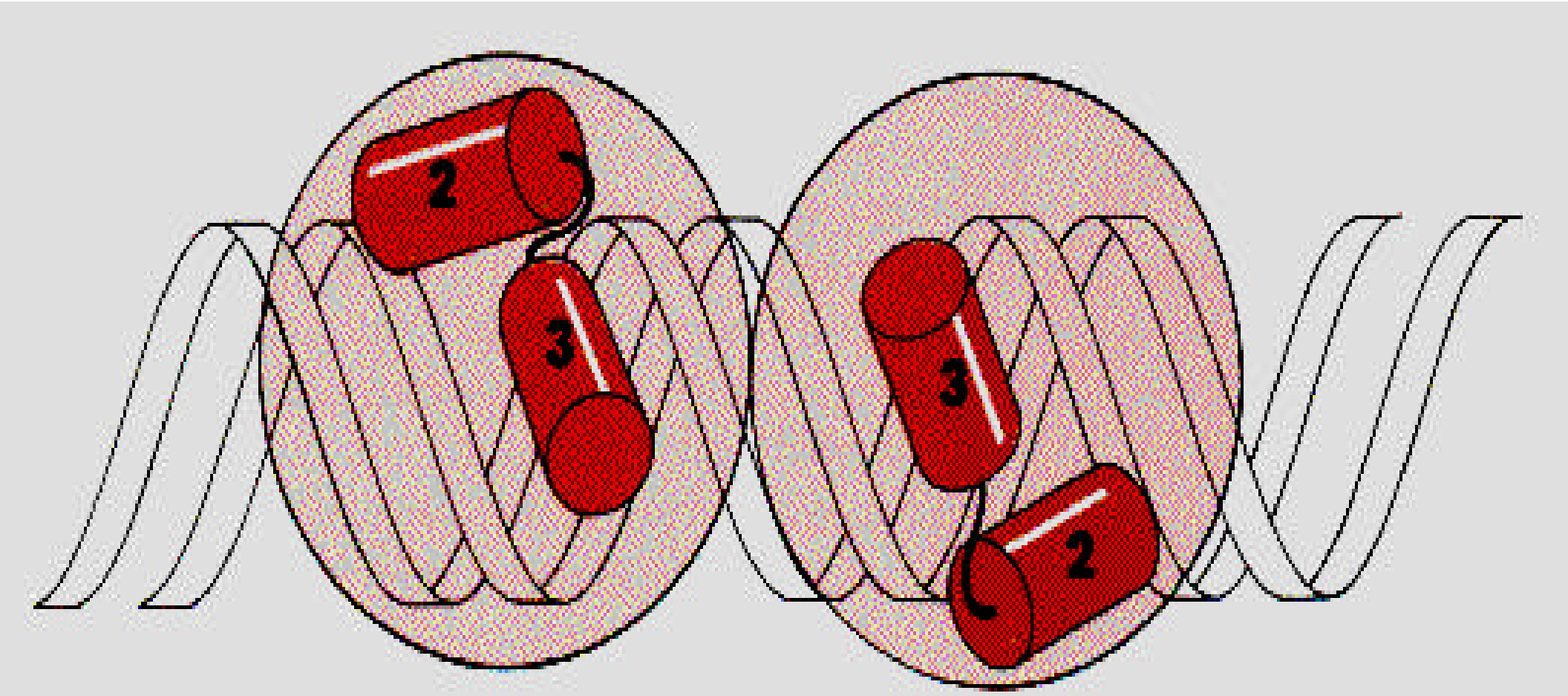
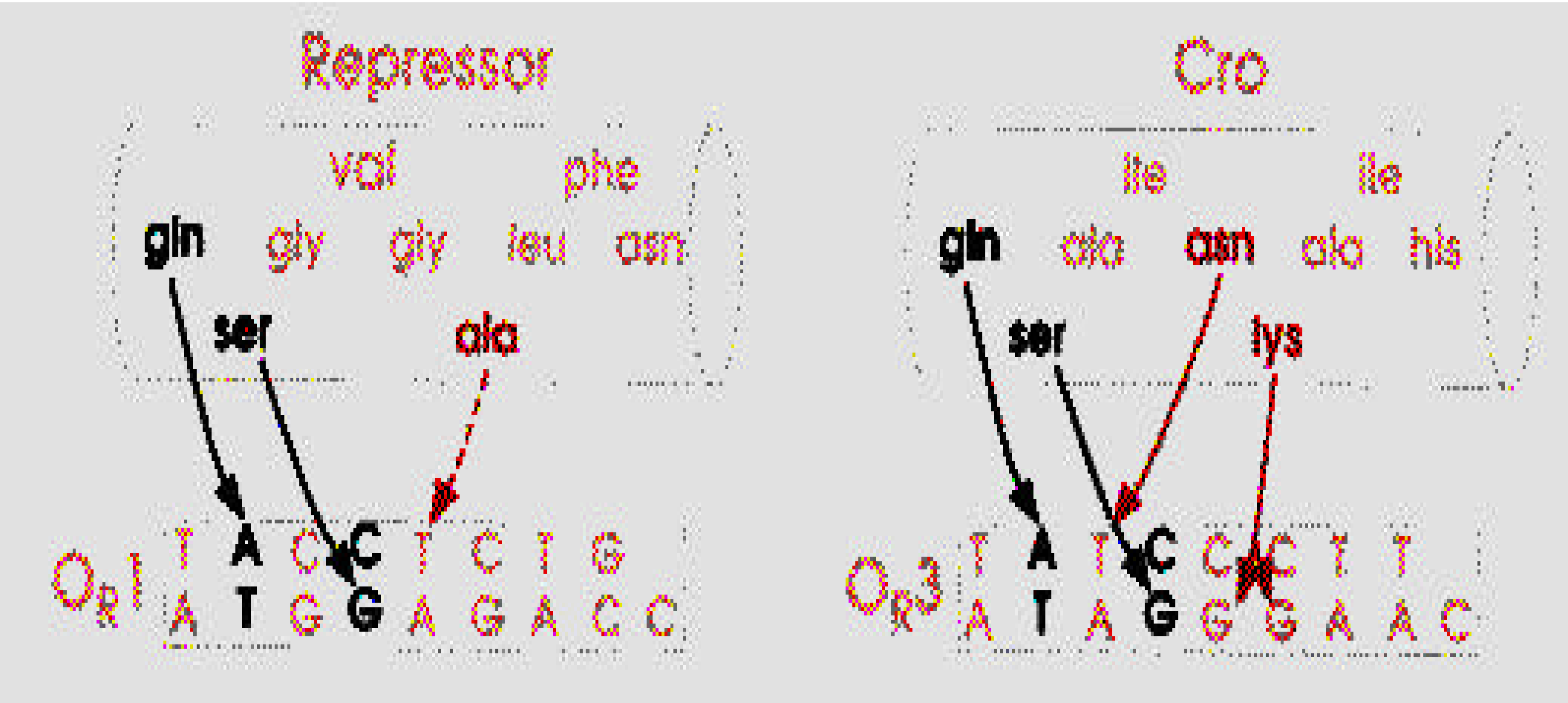



Figure 2.7. Bihelical units on the operator. Two bihelical units are symmetrically positioned on an operator site. Bihelical units are also called helix-turn-helix motifs.

# Repressor / Cro Binding



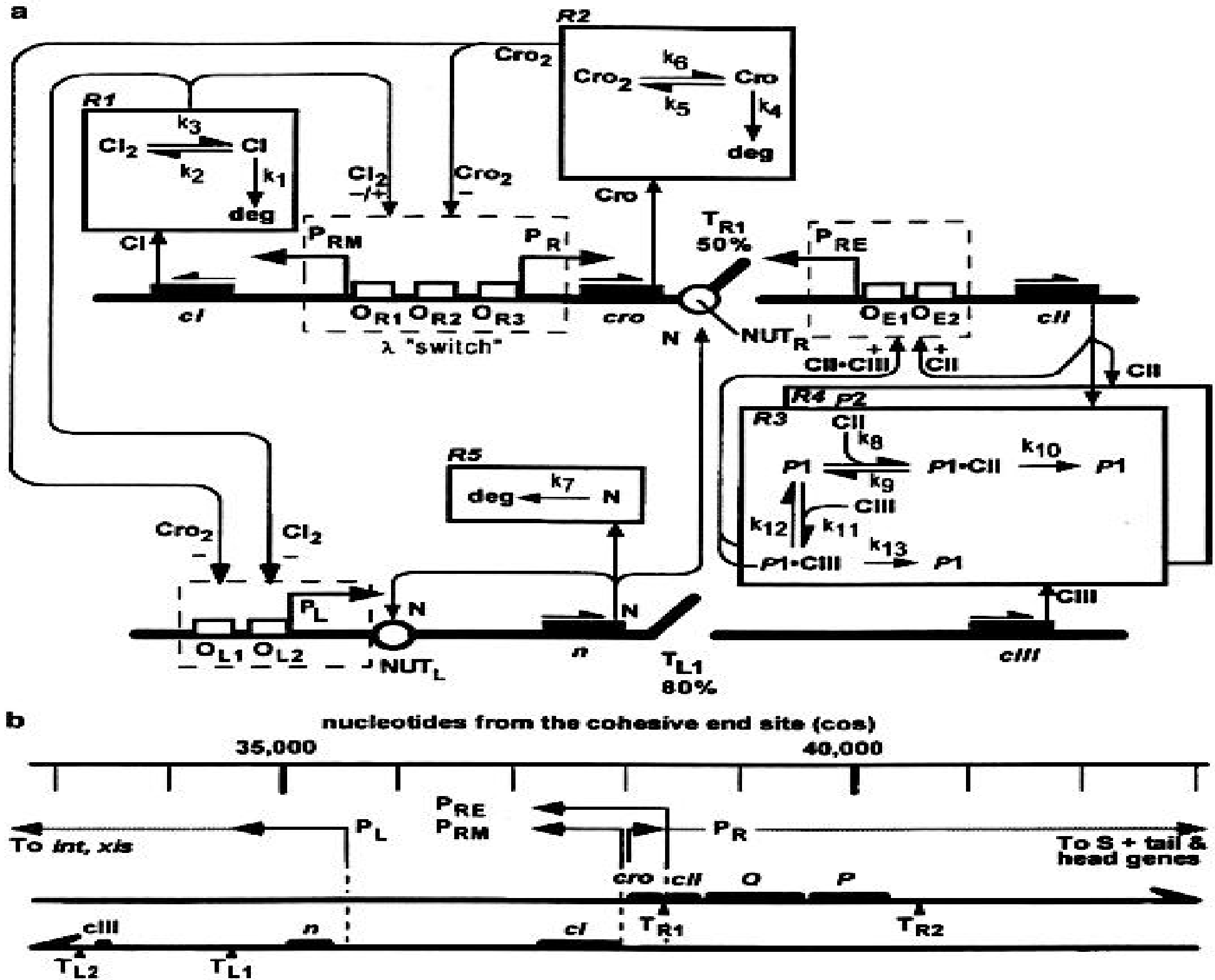
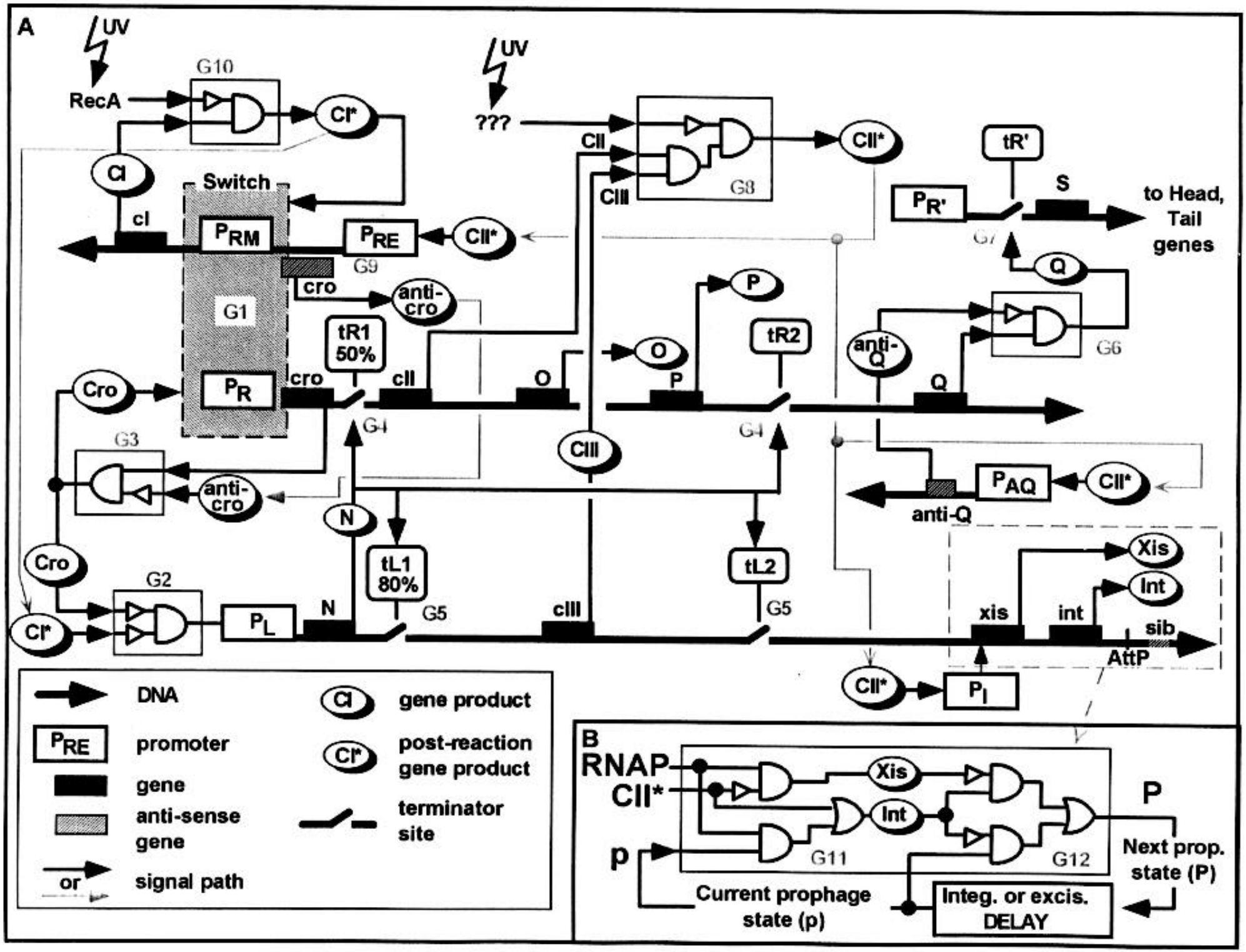
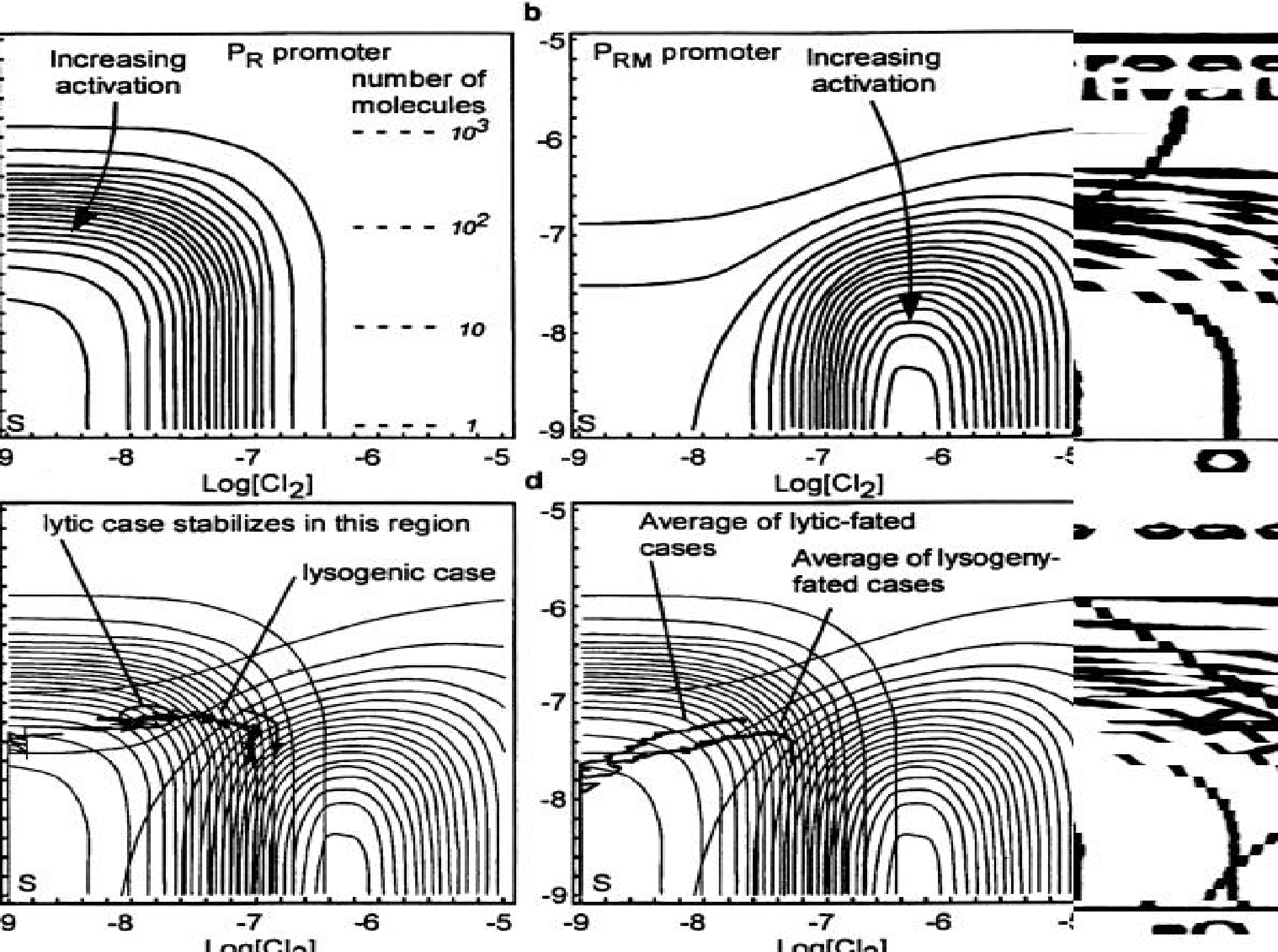
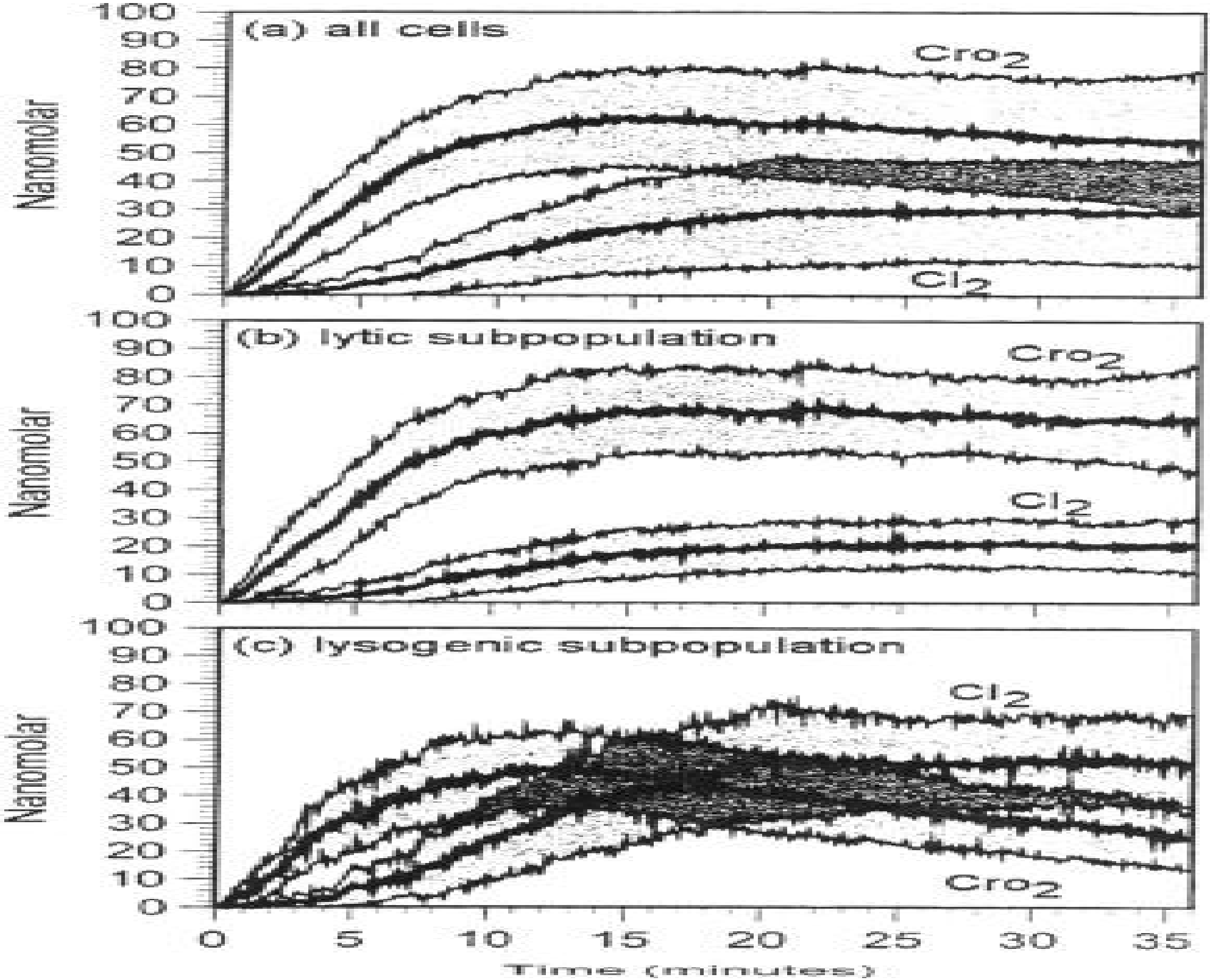
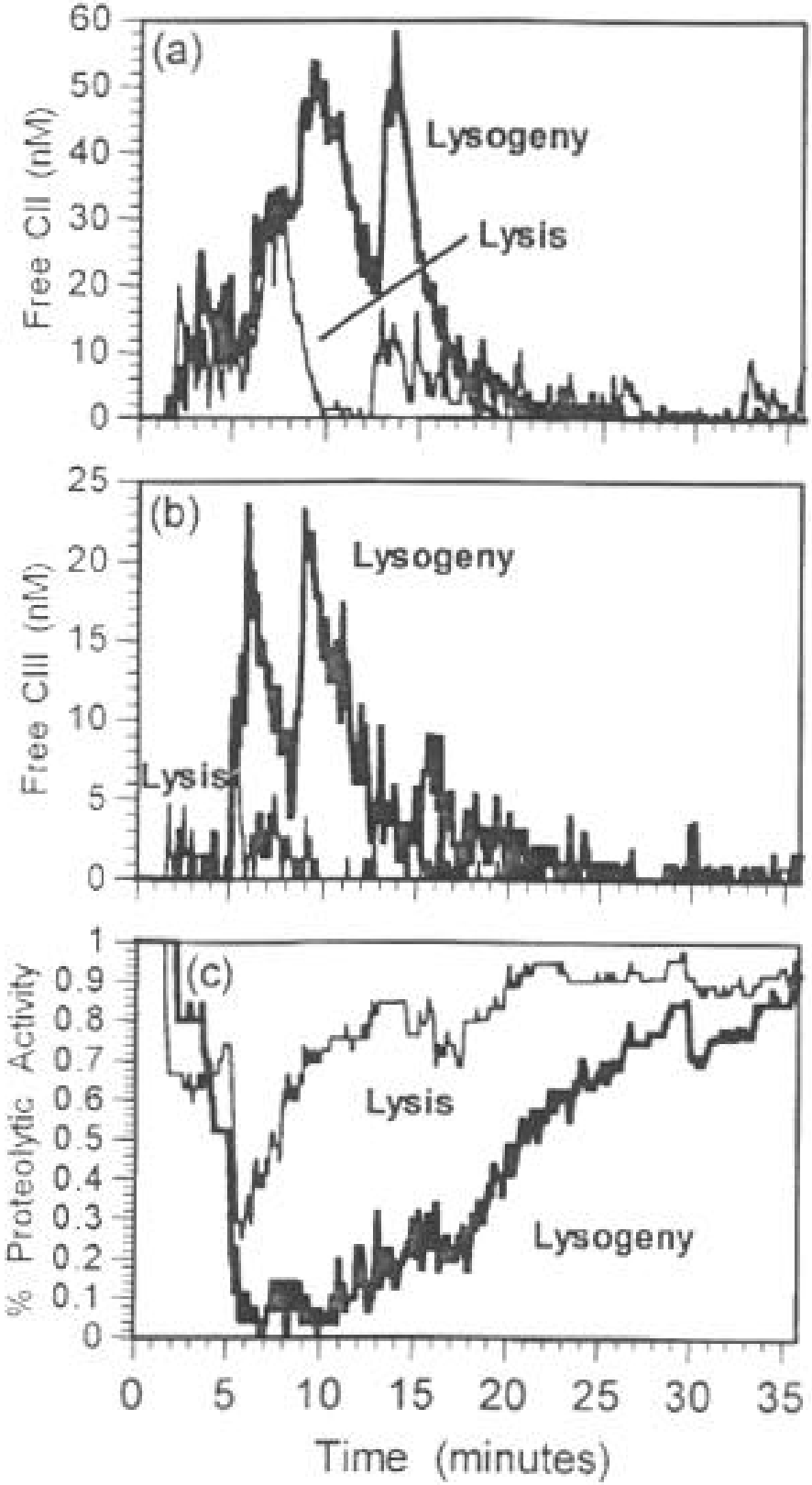

a
R2
Cro2
k6
Cro
k5
k4
deg
R1
k3
Cl2
Cl
k2
k1
deg
Cl2
Cro2
Cro
TR1
50%
PRE
PRM
PR
Cl
cI
OR1 OR2 OR3
cro
λ "switch"
N
NUTR
OE1 OE2
cII
CII-CIII
CII
CII
R4 P2
R3
CII
k8
k10
P1
P1-CII
P1
k9
CIII
k12
k11
k13
P1-CIII
P1
R5
deg
k7
N
Cro2
Cl2
PL
N
N
OL1 OL2
NUTL
n
TL1
80%
CIII
cIII
b
nucleotides from the cohesive end site (cos)
35,000
40,000
PRE
PRM
PL
PR
To int, xis
To S + tail &
head genes
cro
cII
O
P
cIII
n
cI
TR1
TR2
TL2
TL1

Figure 6


A
UV
RecA
G10
CI*
CI
Switch
cI
PRM
PRE
CII*
G9
cro
anti-cro
G1
tR1 50%
PR
cro
cII
O
Cro
G4
G3
anti-cro
N
tL1 80%
G5
cIII
Cro
G2
CI*
PL
N
UV
???
CII
CIII
G8
CII*
tR'
PR'
S
to Head, Tail genes
G7
Q
P
tR2
O
P
anti-Q
Q
G6
G4
PAQ
CII*
anti-Q
tL2
G5
Xis
Int
xis
int
sib
AttP
CII*
PI
DNA
promoter
gene
anti-sense gene
signal path
gene product
post-reaction gene product
terminator site
B
RNAP
CII*
p
Xis
Int
G11
G12
P
Next prop. state (P)
Current prophage state (p)
Integ. or excis. DELAY


b
Increasing activation
$P_R$ promoter
number of molecules
$10^3$
$10^2$
10
1
S
-9
-8
-7
-6
-5
Log[$Cl_2$]
$P_{RM}$ promoter
Increasing activation
d
lytic case stabilizes in this region
lysogenic case
Average of lytic-fated cases
Average of lysogeny-fated cases


(a) all cells
(b) lytic subpopulation
(c) lysogenic subpopulation
Cro2
Cl2
Nanomolar
Time (minutes)
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80
90
100



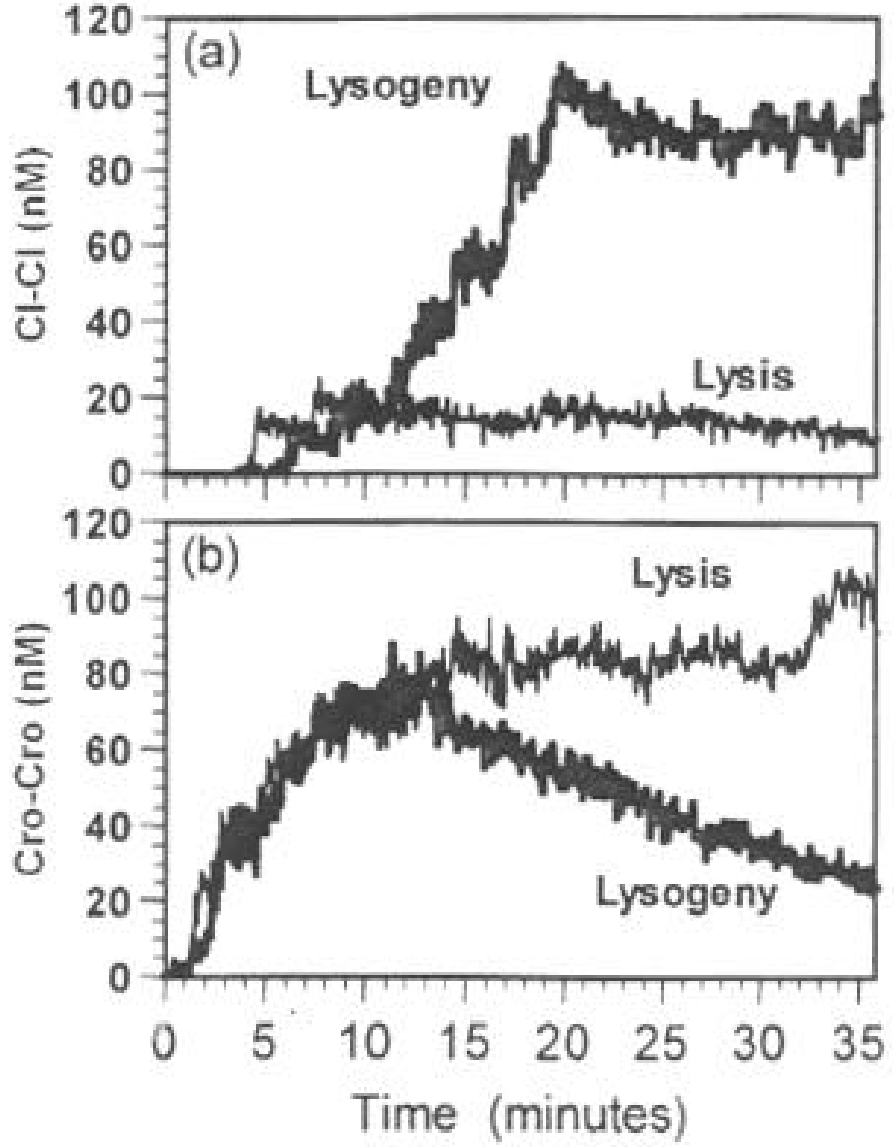




FIGURE 5.—Time evolution of Cro and CI dimer concentrations for the same two simulation runs at MOI 6 as Figure 4. For the lysogenic case (bold), the high CII concentration after 6 min (Figure 4a) leads to the accumulation of $CI_2$ (a) and cessation of Cro production (b). Dilution and degradation causes $Cro_2$ concentration to decline thereafter. For the lytic case, in contrast, the initial burst of CII is not sustained (Figure 4a) so that $P_{RE}$ is not significantly activated and CI production is negligible (a). $Cro_2$ growth begins immediately after infection (b) and, in lytic cases, continues building until it represses